

What is claimed is:

1. A method to detect a nucleotide, comprising:
 - a) restraining movement of a nucleic acid molecule attached to a single particle using a restriction barrier located within a first channel;
 - b) contacting the nucleic acid molecule with an exonuclease to release the nucleotide, wherein the nucleotide is a terminal nucleotide; and
 - c) identifying the released nucleotide by associating the released nucleotide with a surface enhanced Raman spectroscopy-active surface, irradiating the released nucleotide with a detection laser beam and measuring Raman emission from the irradiated nucleotide, thereby detecting the nucleotide.
2. The method of claim 1, wherein the restriction barrier comprises a plurality of walls.
3. The method of claim 2, wherein the restriction barrier comprises a first angled wall and a second angled wall positioned relative to the first angled wall to capture the single particle having the surface with the attached nucleic acid molecule.
4. The method of claim 1, wherein a gradient force optical trap captures the single particle downstream of the laser beam, transports the single particle upstream of the restriction barrier, and release the single particle.
5. The method of claim 1, wherein a gradient force optical trap captures the single particle downstream of the detection laser beam, the detection laser beam and the restriction barrier are moved downstream of the captured single particle, and the single particle is released.
6. A method to determine a nucleotide sequence of a nucleic acid molecule, comprising:
 - a) restraining movement of a single particle using a restriction barrier located within a first channel, wherein the nucleic acid molecule is attached to the single particle;
 - b) contacting the nucleic acid molecule with an exonuclease to release a terminal nucleotide; and
 - c) identifying a first released nucleotide and a second released nucleotide by irradiating the first released nucleotide and then the second released nucleotide with

light from a detection light source, by associating the first released nucleotide and the second released nucleotide with a surface enhanced Raman spectroscopy-active surface, and measuring Raman emission from the irradiated first released nucleotide and then from the second released nucleotide, thereby determining a nucleotide sequence of the nucleic acid.

7. The method of claim 6, wherein the restriction barrier comprises a plurality of walls.
8. The method of claim 7, wherein the restriction barrier comprises a first angled wall and a second angled wall positioned relative to the first angled wall to capture the single particle having the surface with the attached nucleic acid molecule.
9. The method of claim 6, wherein a gradient force optical trap captures the single particle downstream of the light from the detection light source, transports the single particle upstream of the restriction barrier, and releases the single particle.
10. An apparatus comprising, a first channel comprising a restriction barrier comprising a first angled wall and a second angled wall positioned relative to the first angled wall to form a first opening at least 1 micron in width or diameter and a second opening less than 10 microns in width or diameter, wherein the first opening has a greater width or diameter than the second opening.
11. The apparatus of claim 10, wherein the second opening is less than 1 micron in width or diameter.
12. The apparatus of claim 10, further comprising a light source and a detector to detect a surface enhanced Raman spectroscopy emission of a molecule irradiated by the light source, the first channel in optical communication with the light source and the detector.
13. A system comprising:
 - a) a light source;
 - b) a detector to detect a surface enhanced Raman spectroscopy emission of a molecule irradiated by the light source; and

- c) a first channel in optical communication with the light source and the detector, wherein the first channel comprises a restriction barrier comprising a plurality of walls to restrain movement of a single particle upstream of light emitted by the light source.

14. The system of claim 13, wherein the restriction barrier comprises a first angled wall and a second angled wall positioned relative to the first angled wall to form a first opening at least 1 micron in width or diameter and a second opening less than 10 microns in width or diameter, wherein the first opening has a greater width or diameter than the second opening.

15. The system of claim 14, further comprising a laser light source and a series of lenses to form a gradient force optical trap.

16. The system of claim 15, further comprising a second channel forming a junction with the first channel.

17. The system of claim 16, wherein the restriction barrier is located upstream of the junction of the first channel and the second channel.

18. The system of claim 17, wherein the gradient force optical trap is positioned downstream of the junction of the first channel and the second channel.

19. The system of claim 18, wherein the light source is positioned downstream from the restriction barrier and upstream from the gradient force optical trap.

20. The system of claim 13, wherein a portion of a flow path in optical communication with the detection light source is coated with silver, gold, platinum, copper or aluminum.

21. A method for contacting a first molecule with a second molecule within a microfluidic device, comprising delivering at least one hydrodynamically focused flow through the microfluidic device, wherein the hydrodynamically focused flow brings the second molecule into contact with the first molecule only at a target region of the first molecule.

22. The method of claim 21, wherein the hydrodynamically focused flow has a width of less than 1 micron.

23. The method of claim 21, wherein the first molecule is a nucleic acid molecule.
24. The method of claim 23, wherein the nucleic acid molecule is immobilized on a solid support.
25. The method of claim 24, wherein the nucleic acid molecule is aligned before it is contacted with the second molecule.
26. The method of claim 24, wherein the target region comprises a terminus of the nucleic acid molecule.
27. The method of claim 26, wherein the second molecule is an enzyme.
28. The method of claim 27, wherein the enzyme is an exonuclease.
29. The method of claim 23, wherein after the at least one hydrodynamically focused flow contacts the first molecule at the first target region, the at least one hydrodynamically focused flow is changed to contact the first molecule at a second target region.
30. The method of claim 26, wherein the second molecule is a phosphoramidite nucleic acid.
31. The method of claim 21, wherein the at least one hydrodynamically focused flow is at least partially focused by a protective flow layer between the hydrodynamically focused flow and a surface of the first channel.
32. The method of claim 31, wherein the first molecule is a nanotube.
33. The method of claim 32, wherein the second molecule is a label.
34. The method of claim 21, further comprising a protective flow that at least partially inhibits the second molecule from contacting a surface of the microfluidic device.
35. The method of claim 34, wherein the protective flow completely blocks non-specific binding of the second molecule to a surface of the microfluidic device.
36. The method of claim 34, wherein the protective flow comprises an undetectable amount of the first molecule or the second molecule.

37. The method of claim 34, further comprising a second hydrodynamically focused flow that at least partially inhibits the second molecule from contacting a second surface of the microfluidic device.
38. The method of claim 34, wherein the protective flow has a diameter or width of less than 1 micrometer.
39. The method of claim 34, wherein the first molecule is stretched before the first molecule is contacted by the second molecule.
40. The method of claim 34, wherein the at least one hydrodynamically focused flow is at least partially focused by a first protective flow layer that surrounds the hydrodynamically focused flow.
41. The method of claim 34, wherein the at least one hydrodynamically focused flow is inhibited from contacting a surface of the microfluidic device by the protective flow.
42. A method to inhibit non-specific binding in a microfluidic device, comprising
 - a) immobilizing a first molecule on a surface of the microfluidic device;
 - b) stretching the first molecule;
 - c) delivering at least one protective flow through the microfluidic device; and
 - d) delivering at least one hydrodynamically-focused delivery flow through the microfluidic device, wherein the delivery flow has a second molecule suspended therein, and wherein the protective flow inhibits the delivery flow from contacting the surface of the microfluidic device as the first molecule contacts the second molecule.
43. The method of claim 42, wherein the first molecule is a nucleic acid molecule.
44. The method of claim 43, wherein the nucleic acid molecule is aligned before it is contacted with the second molecule.
45. The method of claim 44, wherein the at least one hydrodynamically focused flow contacts the nucleic acid molecule at a terminus of the nucleic acid molecule.